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The purpose of this research is to develop a non-invasive predictor of malignancy in breast tumors using novel magnetic resonance imaging (MRI) techniques. The hypothesis is that the spatial distribution of microvasculature around a breast lesion is specific for malignancy and can be reliably measured by a completely non-invasive MRI method.
This hypothesis is being tested by:
1) The design and construction of ultra-high gradient coils for MRI.
2) The implementation of advanced MRI pulse sequences for mapping of microvascular parameters
3) The correlation of MRI-derived vascular parameters (diffusion and perfusion) with histological parameters (tumor grade and microvessel density) in an animal model of human breast cancer.

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FOREWORD

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_Paula Gareau_____
PI - Signature   Date

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Introduction

X-ray mammography is currently the clinically accepted modality for breast cancer screening. Mammography, however, is particularly unreliable and recent trials have shown that 70-80% of mammographically indeterminate lesions that progress to surgical biopsy are benign. The development of accurate, non-invasive imaging tests with low false negative rates would allow reduction of the two step process of surgical biopsy followed by surgical lesion removal to a single step, as well as providing a tool for guiding treatment decisions. It may be possible to achieve this through the development of non-invasive imaging techniques that measure vascular parameters of tumors (for example, blood flow, blood volume, and permeability or microvessel density). Tumor progression is known to depend on the ability to stimulate the growth of new blood vessels that supply nutrients and oxygen to the expanding tumor. This process is known as angiogenesis. It has been hypothesized that the characteristic new vessel growth associated with tumor angiogenesis should allow differentiation between malignant and benign breast lesions. The purpose of this research is to develop a non-invasive predictor of malignancy through the design and preliminary validation of a new form of magnetic resonance imaging (MRI) called the IntraVoxel Incoherent Motion (IVIM) method that utilizes high main magnetic field strength and ultra-strong magnetic field gradients. This is compared with vascular parameters derived from dynamic contrast enhanced MRI.

Body

Hardware Development

A high strength gradient coil has been designed. The prototype coil was built with an inner diameter of 5cm, which is ideal for mice. A second gradient coil has now been built that is more suitable for rats. This coil has an inner diameter of 24cm, which can accommodate a rat plus the equipment used to monitor the animal. The maximum gradient strength achievable with this coil is 300mT/m, which is almost ten times greater than typical clinical systems and is sufficient for the high-resolution imaging experiments proposed in this grant. This coil has been implemented at 1.5 Tesla (T) using a clinical research scanner. A specialized radiofrequency surface coil was designed and

constructed to allow the acquisition of high quality MR data with very high signal to noise.

Pulse Sequence Development

An IVIM pulse sequence has been designed based on a standard diffusion-weighted imaging sequence. The sequence consists of a spin echo, echo planar imaging sequence with the addition of strong magnetic field gradients that cause the contrast in the images to be weighted based on the diffusion of water in the microvascular and extra-vascular compartments. We have incorporated a phase correction method that allows post-acquisition correction of the images for small-scale motion of the object being imaged.

A computer program has been written in an interactive windows environment that allows analysis of the diffusion-weighted images obtained with the IVIM pulse sequence. The program allows calculation of the diffusion coefficients either for each pixel in the images, or for the average values in a region of interest. Validation of the program has been performed using simulated data..

Implementation of Mammary Tumor Model in Rats

A mammary tumor model has been implemented in female Sprague-Dawley rats using N-ethyl-N-nitrosourea (ENU). ENU is known to be a potent carcinogen that induces well vascularized mammary tumors in rats. This tumor model was chosen because a range of benign and malignant tumors develops depending on the dose of ENU administered and because the tumor morphology and angiogenic behavior closely mimics breast tumors in humans. Mammary tumors developed in 28/30 rats administered a high dose of ENU (180 mg/kg) and in 6/30 rats administered a low dose of ENU (45 mg/kg).

Validation of Microvascular Imaging

Initial validation studies of the IVIM sequence were performed using phantoms that are spherical containers filled with water and acetone. The results obtained (Appendix I) agree well with those found in the literature.

High signal to noise ratios (SNR) are required in the IVIM images in order to observe the microvascular component of the measured signal. The average SNR in the IVIM images, obtained using the custom-built RF coil, is approximately 400, which we predict is sufficient for this measurement.

Preliminary results (Appendix II) show a fast decay component. The magnitude of the diffusion coefficient for this component is in on the order of that expected for the microvascular blood flow. To verify that the IVIM component is related to microvascular blood flow some animals were imaged before and after sacrifice. This fast decay component disappears after cessation of blood flow. These results suggest that the IVIM method is capable of measuring the tumor microvasculature.

Dynamic Contrast Enhanced MRI (DCE-MRI)

For DCE-MRI we extended a previously developed magnetization prepared 3D SPGR imaging sequence. Rats were imaged before and during bolus contrast agent injection (Gadolinium-DTPA). Axial slices containing both the tumor and the aorta are obtained to allow for determination of the tissue and blood enhancement curves. The tumor blood flow, blood volume and permeability-surface area product were obtained from kinetic analysis of the dynamic tumor enhancement responses.

The accuracy of the DCE-MRI technique was assessed by comparing DCE-MRI results with computed tomography (CT) data. Dynamic enhanced CT was performed on the same tumor within 24 hours after the DCE-MRI scanning. The CT derived vascular parameters (blood flow, blood volume and mean transit time or permeability) are used as a gold standard against which to compare the DCE-MRI derived vascular parameters.

Both IVIM and DCE-MRI images were obtained from each tumor.

Histologic Analysis

Tumors were removed and prepared for histologic analysis. Formalin-fixed tumors were sliced with guidance form the MR images and digital images taken during the dissection performed to obtain histological tissue slices that correspond to the images slices from which MR measurements are made. The tumors have been resected and a histopathologic analysis was performed to determine the microvessel density and spatial distribution, tumor type and tumor grade. The quantitative MRI-derived vascular parameters will be correlated with the histopathologic tumor grade and other markers of tumor angiogenic activity.

Key Research Accomplishments

- Design of a mouse-sized gradient coil with gradient strengths that exceed 2000°mT/m. (Approximately 50 to 100 times higher than typical clinical MRI systems.)
- Design and construction of a gradient coil with an inner diameter of 24 cm, which is large enough to accommodate a rat and animal monitoring equipment. Maximum gradient strengths achieved with this coil are 300 mT/m.
- Implementation of a fast IVIM pulse sequence that allows acquisition of diffusion weighted images with high signal to noise.
- Design and coding of a computer program that allows automated analysis of the diffusion weighted images for the calculation of diffusion coefficients.
- Validation of the MR pulse sequence analysis program using simulated data and images acquired from substances with known diffusion coefficients.
- Implementation of a breast tumour model in rats that produces tumours with a range of malignancies from benign to malignant.
- Acquisition of high signal to noise ratio, *in vivo,* diffusion weighted images of tumours in the rats using the IVIM method.
- Acquisition of in vivo dynamic contrast enhanced images of tumors
- Verification of three unique diffusion rates in the pre-sacrifice tumours.
- Verification that the highest diffusion rate is related to the flow of blood in the microvasculature.

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Reportable Outcomes

Presentations and Publications

The design and construction of the gradient coils resulted in three peer-reviewed publications and two conference presentations.

Manuscripts

1. Chronik BA, Rutt BK. Constrained Length Minimum Inductance Gradient Coil Design. *MRM* **44**:955-963 (2000).

2. Maier CF, Nikolov HN, Chu KC, Chronik BA, Rutt BK. Practical design of a high-strength breast gradient coil. *MRM* **39**:392-401 (1998).

3. Chronik BA, Rutt BK. Constrained length minimum inductance gradient coil design. *MRM* **39**:270-278 (1998).

Conference Presentations

1. Chronik BA, Alejski A, Rutt BK. A 2000mT/m multilayer gradient coil for mouse imaging. *Proceeding of the International Society for Magnetic Resonance in Medicine* **7**:496 (1999).

2. Rutt BK, Maier CVF. Design and construction of gradient coils for diffusion breast imaging. Invited presentation at the *Society of Magnetic Resonance Breast Imaging Workshop*, Washington D.C., June 24-25, 1995.

The preliminary results of the acquisition and analysis of the images, acquired from the animal tumour model, were presented at the Era of Hope Meeting, Atlanta, June 8-12, 2000.

Friesen-Waldner L, Gareau P, Chronik B, Rutt B. Magnetic resonance imaging techniques for the examination of tumour vasculature. *Era of Hope, Department of Defense Breast Cancer Research Program Meeting* **1**:191 (2000).

Additional results will be presented at the Era of Hope Meeting in Orlando, Florida in September 2002.

At least two additional papers are planned for submission.

Conclusions

We designed a mouse-sized gradient coil and a gradient coil large enough to accommodate rats and animal monitoring equipment. Both coils achieve gradient strengths significantly higher than typical clinical MRI systems. Based on wire density and power dissipation considerations, we concluded that small coils with high gradient strengths, such as those required for high resolution IVIM imaging, require multi-layer configurations. The design for the coils constructed here should be scalable to a breast-sized coil. Such a coil will allow imaging of the human breast at resolutions that, until now, have been unattainable, thus allowing visualisation of very small lesions within the breast.

The IVIM pulse sequence was implemented on our 1.5T clinical MRI system. We are able to acquire IVIM images with high signal to noise ratios (at least 200). A computer program was written to analyse the IVIM images. The pulse sequence and analysis program were validated using substances with known diffusion coefficients.

An animal tumour model was implemented, and IVIM images of the tumours were acquired. We have shown that three diffusion rates exist in the tumours and that, in order to properly separate and accurately classify all three components, the diffusion decay curve must be sampled to a maximum b-value of at least 5000 s/mm^2. A multi-exponential fitting algorithm, such as NNLS, capable of more than bi-exponential fitting, must be used to determine accurately the diffusion coefficients from the decay curves. The largest of the three diffusion rates detected by the IVIM method is absent after cessation of blood flow. For this reason, we believe that this large diffusion rate arises from the tumour vasculature. Our results suggest that the tools developed here should be able to measure non-invasively the microvasculature in breast tumours. This would allow diagnosis of breast lesions *in vivo* and would also provide a means of following the effects of treatments, particularly treatment with anti-angiogenic pharmaceuticals.

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Appendix I

The IVIM method and the analysis program developed for this grant were tested with two substances — water and acetone - whose diffusion coefficients are well documented. The theoretical and experimentally determined results are shown below for diffusion encoding along six different directions:

Water:

The theoretical value of the diffusion coefficient of water is between 2.12×10^{-3} and 2.63×10^{-3} mm^2/s at 21.7 C°.

| Diffusion Encoding Direction	Diffusion Coefficient (mm^2/s)
xy	$2.03 \pm 0.02 \times 10^{-3}$
-xy	$2.10 \pm 0.09 \times 10^{-3}$
xz	$2.08 \pm 0.10 \times 10^{-3}$
-xz	$1.97 \pm 0.06 \times 10^{-3}$
yz	$2.07 \pm 0.04 \times 10^{-3}$
-yz	$2.07 \pm 0.11 \times 10^{-3}$

Acetone:

The theoretical value of the diffusion coefficient of acetone is between 3.88×10^{-3} and 4.43×10^{-3} mm^2/s at 21.7 C°.

Diffusion Encoding Direction	Diffusion Coefficient (mm^2/s)
xy	$4.47 \pm 0.04 \times 10^{-3}$
-xy	$4.59 \pm 0.05 \times 10^{-3}$
xz	$4.62 \pm 0.03 \times 10^{-3}$
-xz	$4.30 \pm 0.05 \times 10^{-3}$
yz	$4.47 \pm 0.06 \times 10^{-3}$
-yz	$4.55 \pm 0.06 \times 10^{-3}$

Appendix II

Shown below are the bi-exponential diffusion coefficients before (D_A, D_B) and after (D_X, D_Y) cessation of blood flow (euthanasia) in non-necrotic regions of interest in low and high dose tumours.
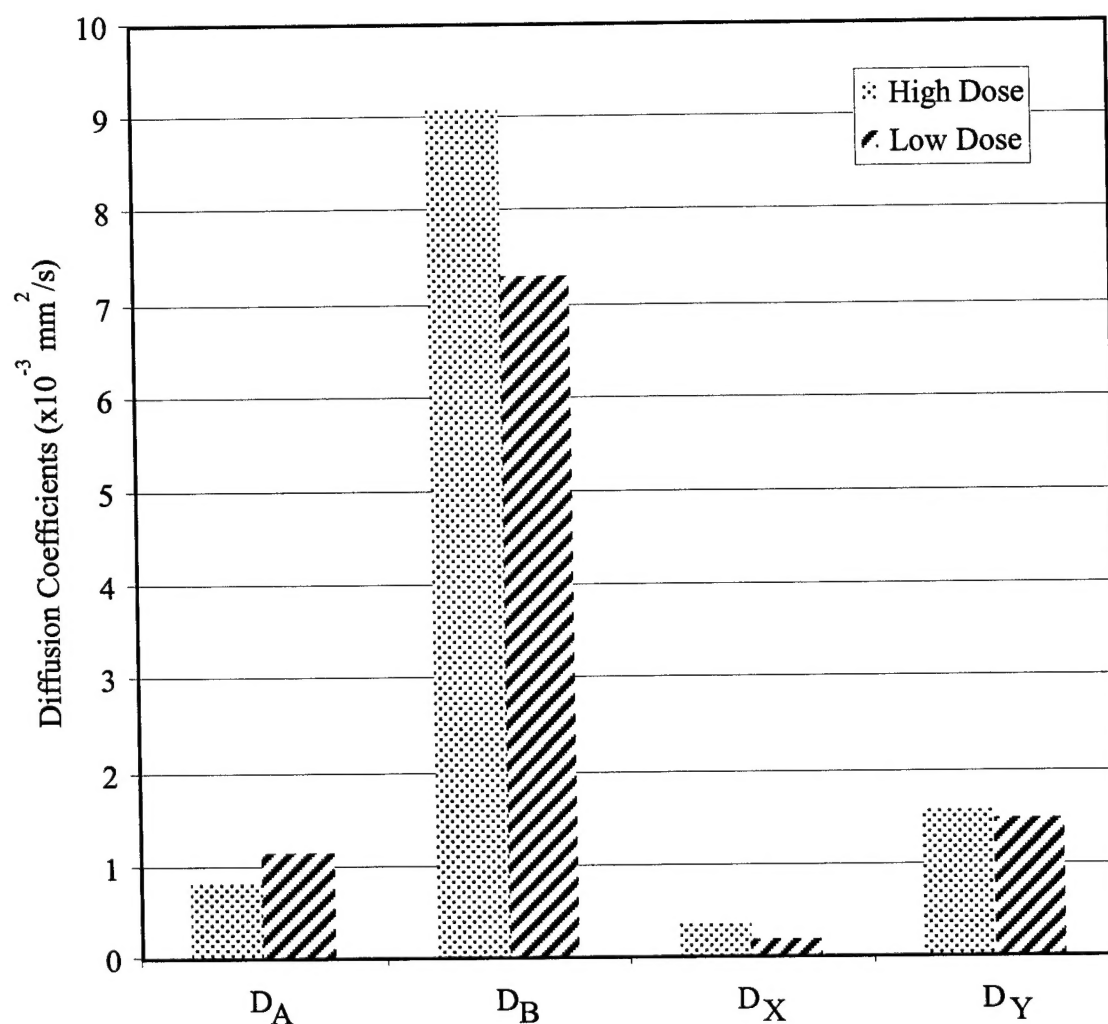
Note that the fast decay component (large diffusion coefficient), D_B, disappears post cessation of blood flow. The presence of a bi-exponential decay post cessation of blood flow may be attributed to different rates of diffusion in the intra- and extracellular spaces. We are currently endeavoring to assign these two diffusion components.